

FIG. 1A

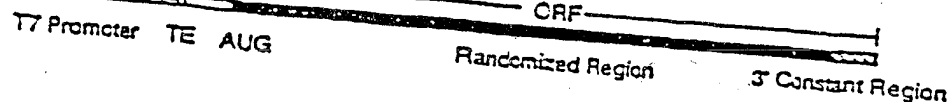


FIG. 1B

Puromycin-tethered oligo is ligated to mRNA (generated from above construct) in the presence of a splint and DNA ligase

note: for short ORFs, this whole template can be made synthetically

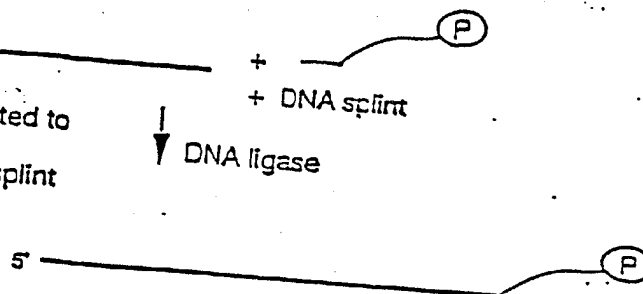
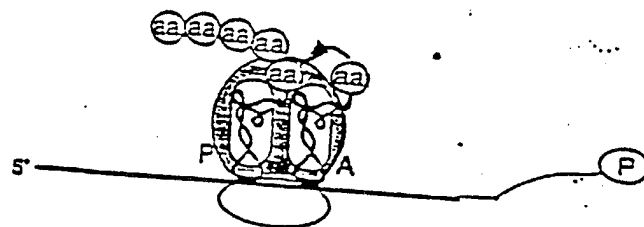
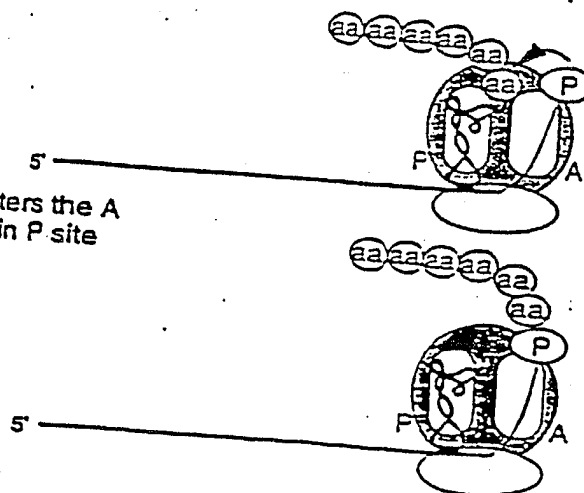


FIG. 1C

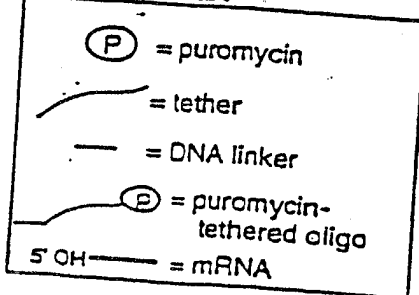
in vitro translation proceeds normally from the 5' to the 3' end of the mRNA



covalently linked puromycin enters the A site and attacks peptidyl tRNA in P site



KEY



Release of RNA-protein fusion with high salt wash of Ribosome

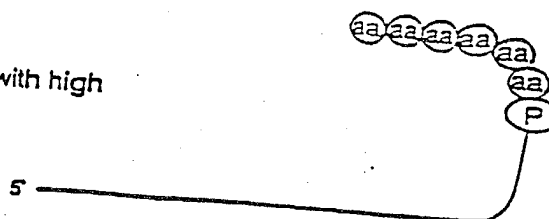
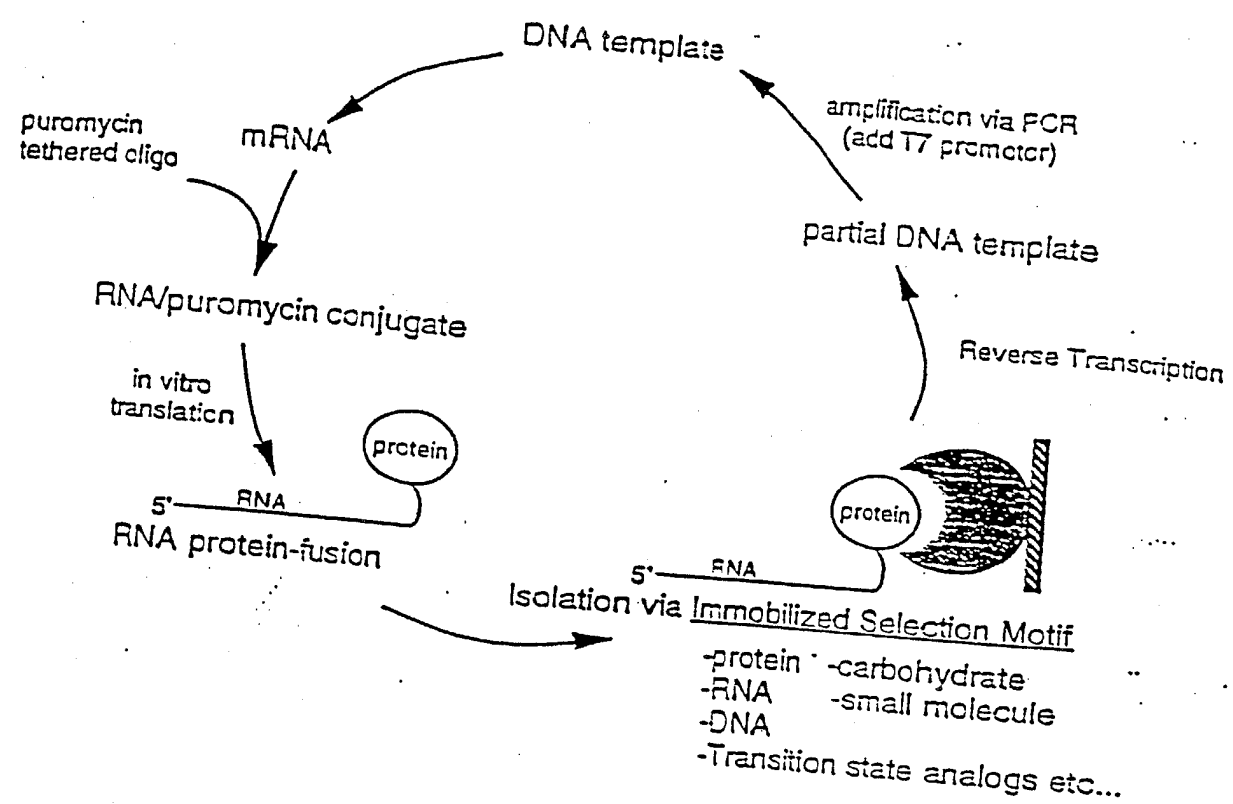
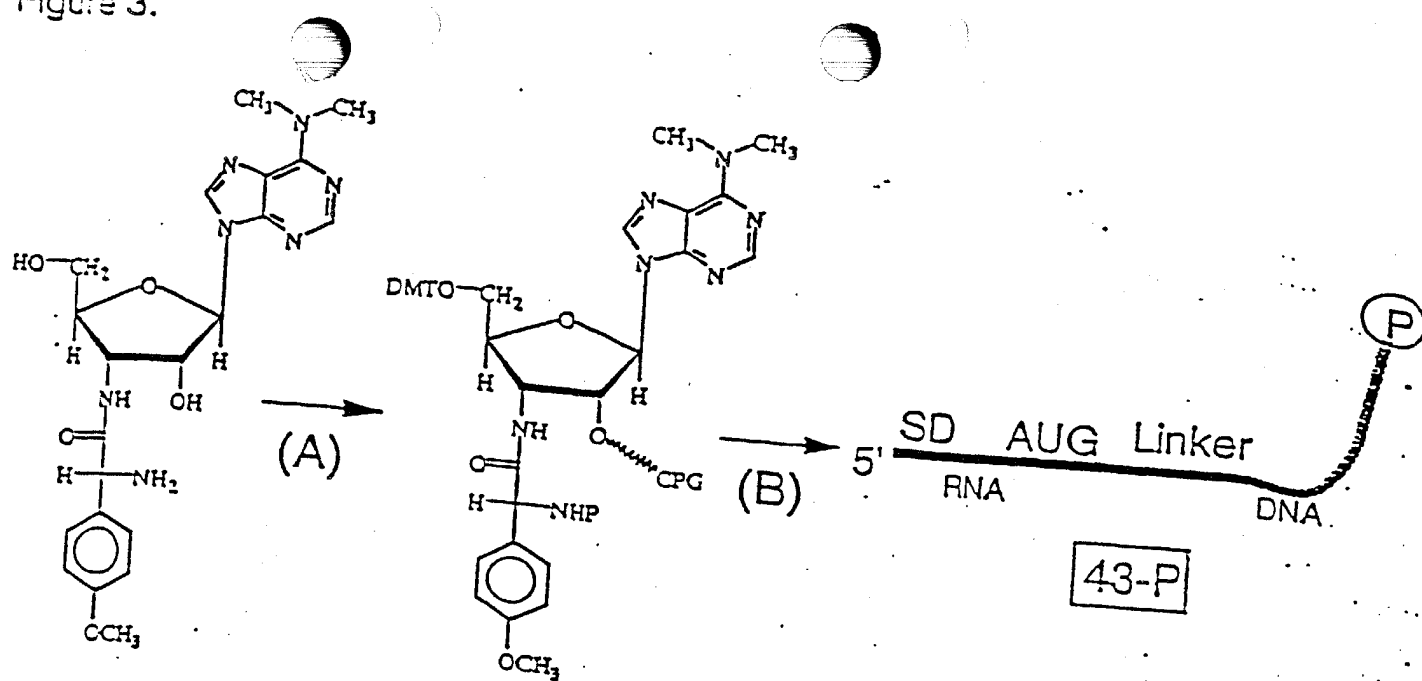


Figure 2. Generalized Protocol for Selection Experiments



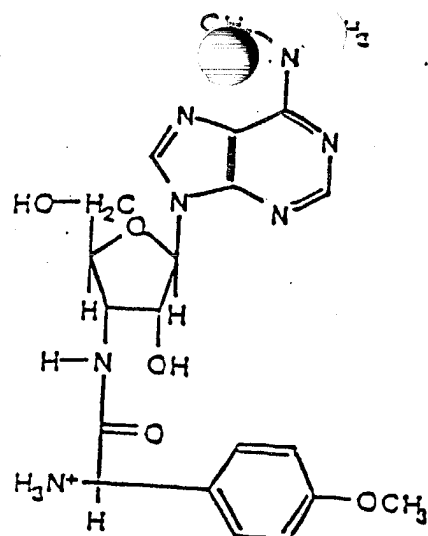
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Figure 3.



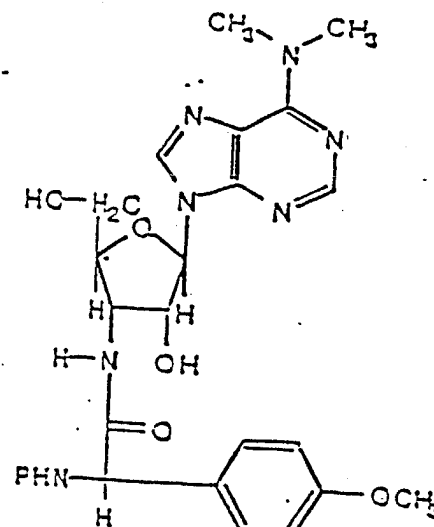
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FIG. 4



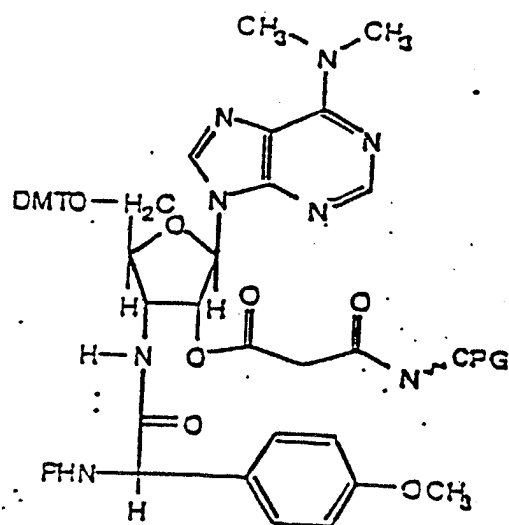
puromycin

protect amino group
with $(\text{CF}_3\text{CO})_2$



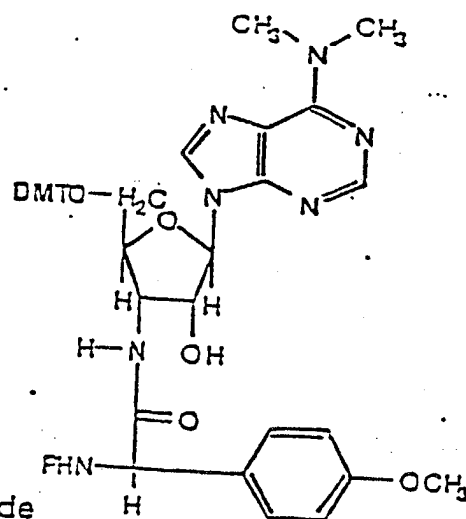
P = protecting group

protect 5'OH with
trimethyl chloride



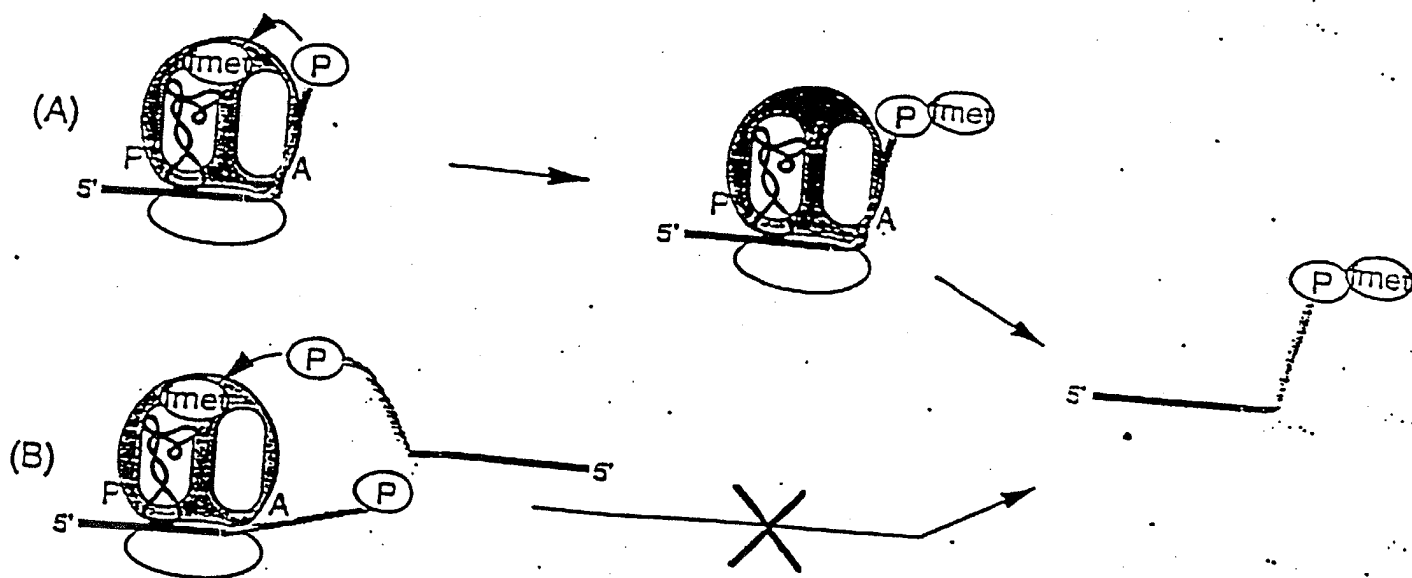
protected, CPG puromycin

link 2'OH to solid
support alkyl amine
CPG with
1) DCC/succinic anhydride
2) *p*-nitrophenol



Use as solid support in
automated DNA synthesizer
-deavage yields 3' puromycin
tethered oligonucleotide

FIG. 5



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Lane
Mg²⁺ [mM] 1 3 4 5 6
3 6 9 12 15 18



FIG. 6A

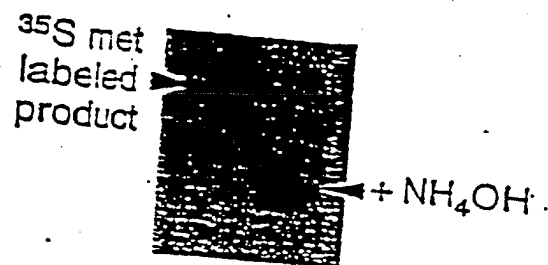


FIG. 6B

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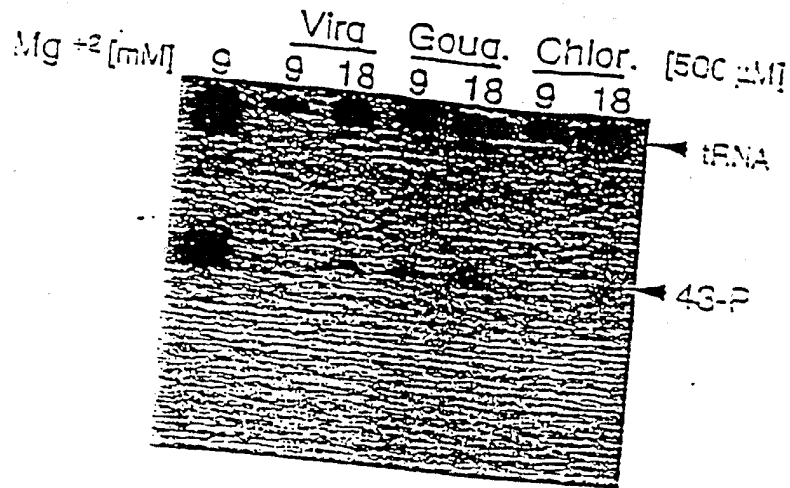


FIG. 6C

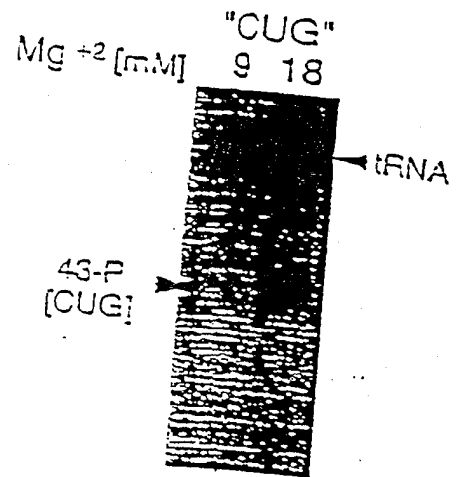


FIG. 6D

Linker (dA) _n	n=27		n=24		n=21		n=18	
Mg ²⁺ [mM]	9	18	9	18	9	18	9	18

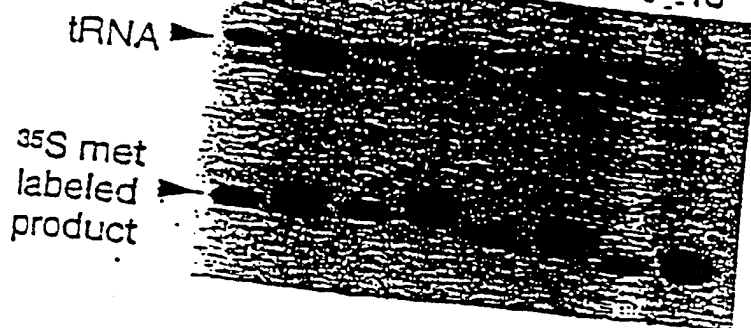


FIG. 6E

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43-P +
25-P +

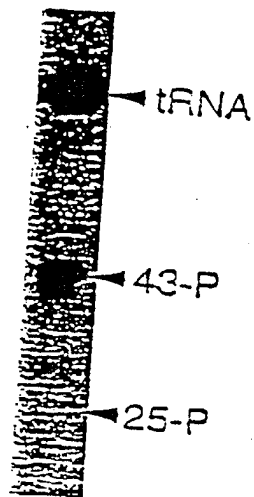


FIG. 6F

43-P + +
13-P - +

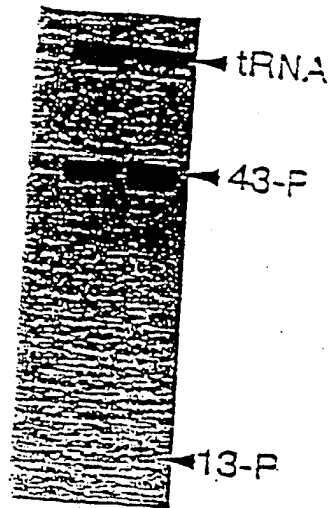


FIG. 6G

30-P - + + -
43-P + - + -



FIG. 6H

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FIG. 7A

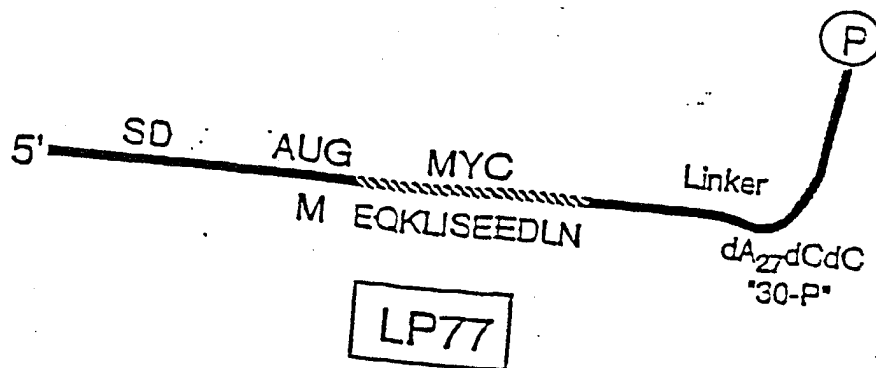


FIG. 7B

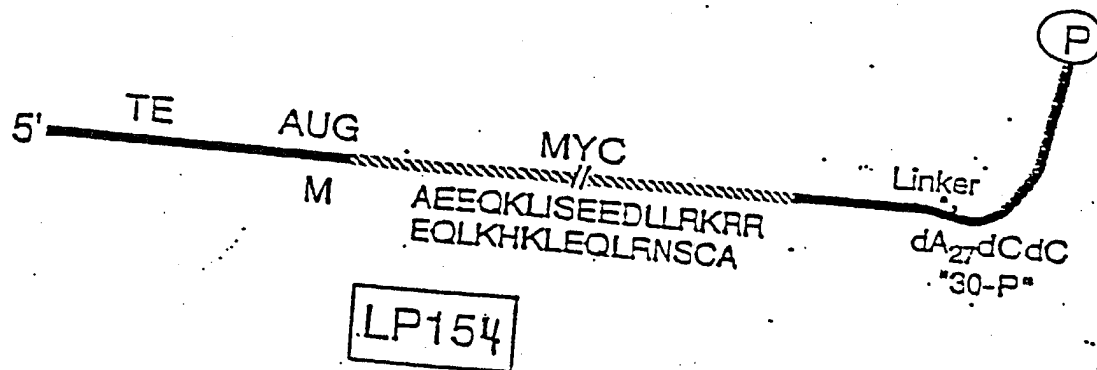
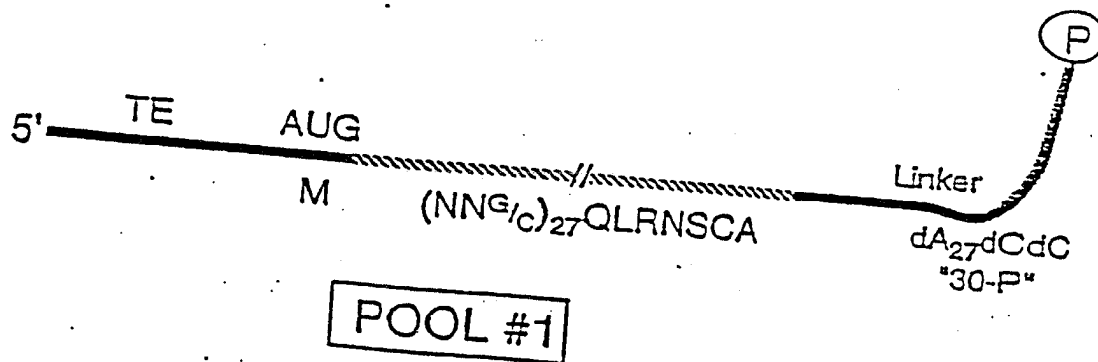


FIG. 7C



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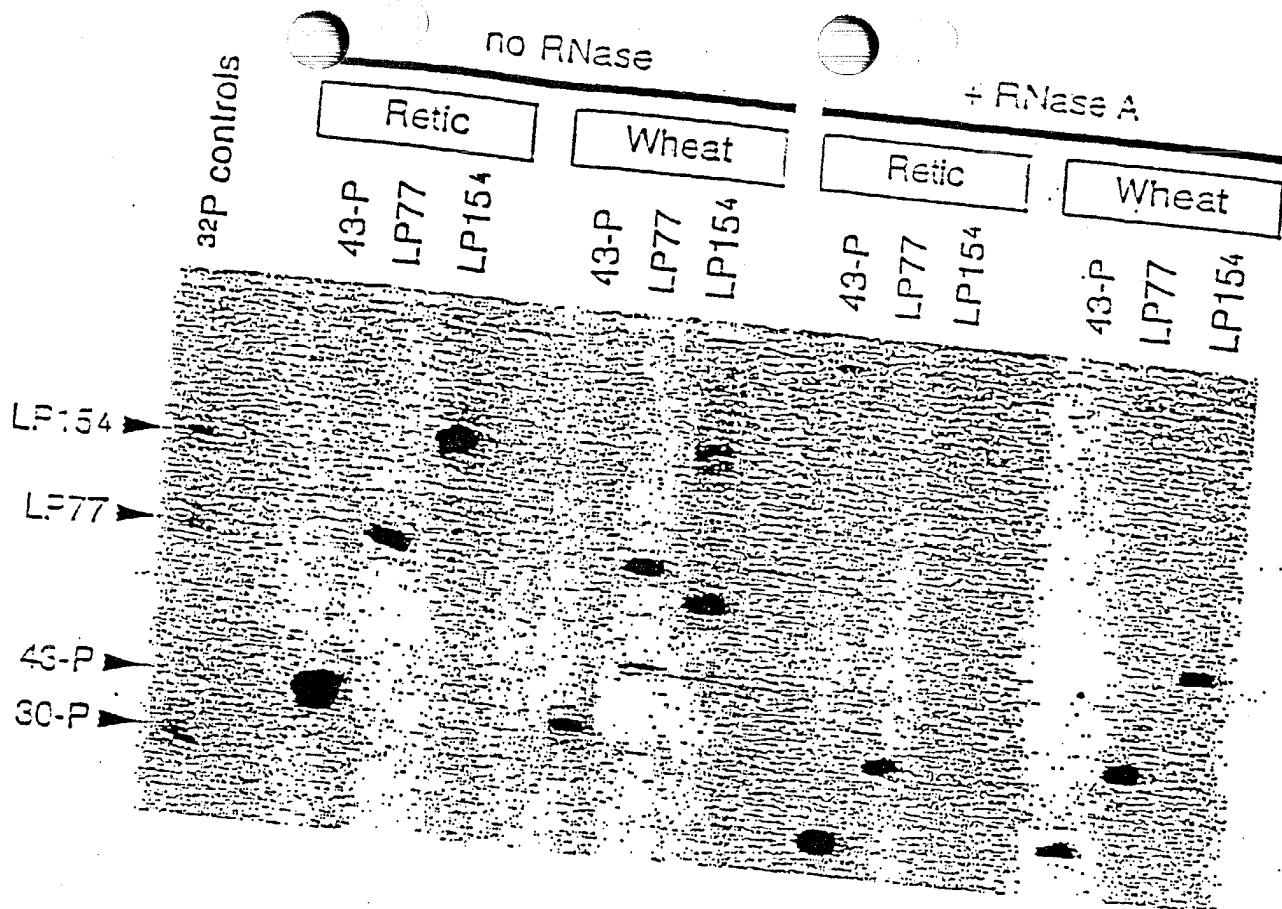


FIG. 8

Effects of RNase A and Proteinase K on fusion

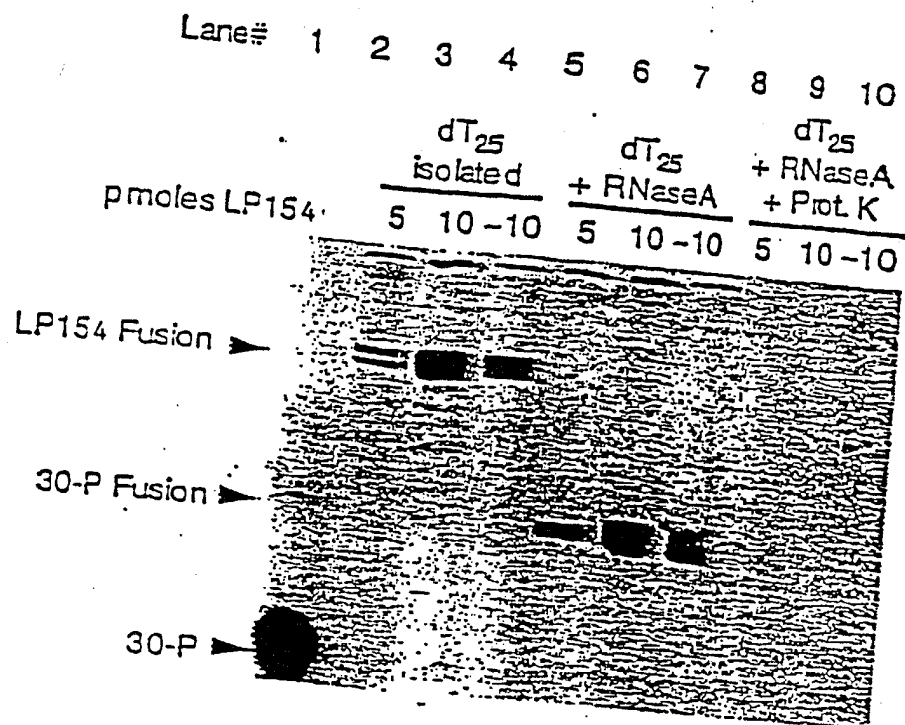
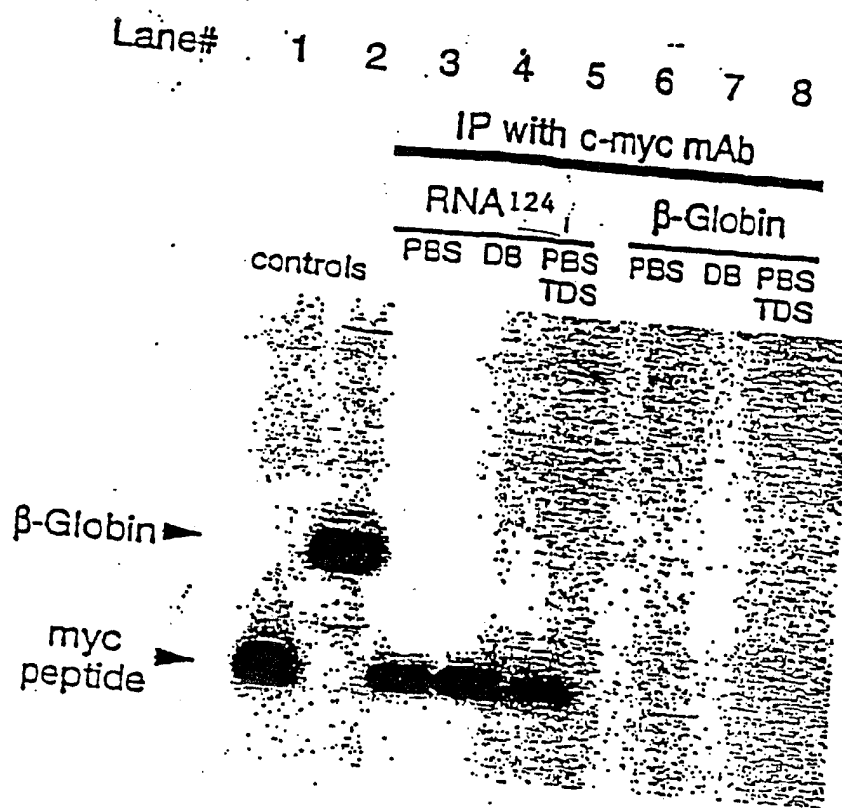
[illegible]

FIG. 10



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FIG. 11

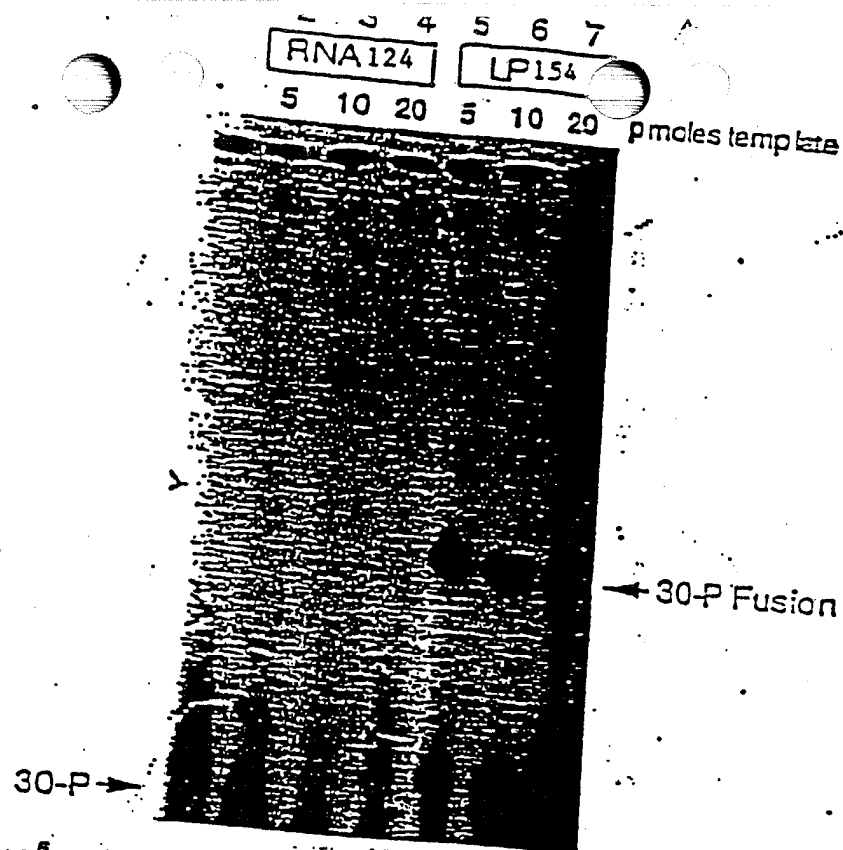


FIG. 12

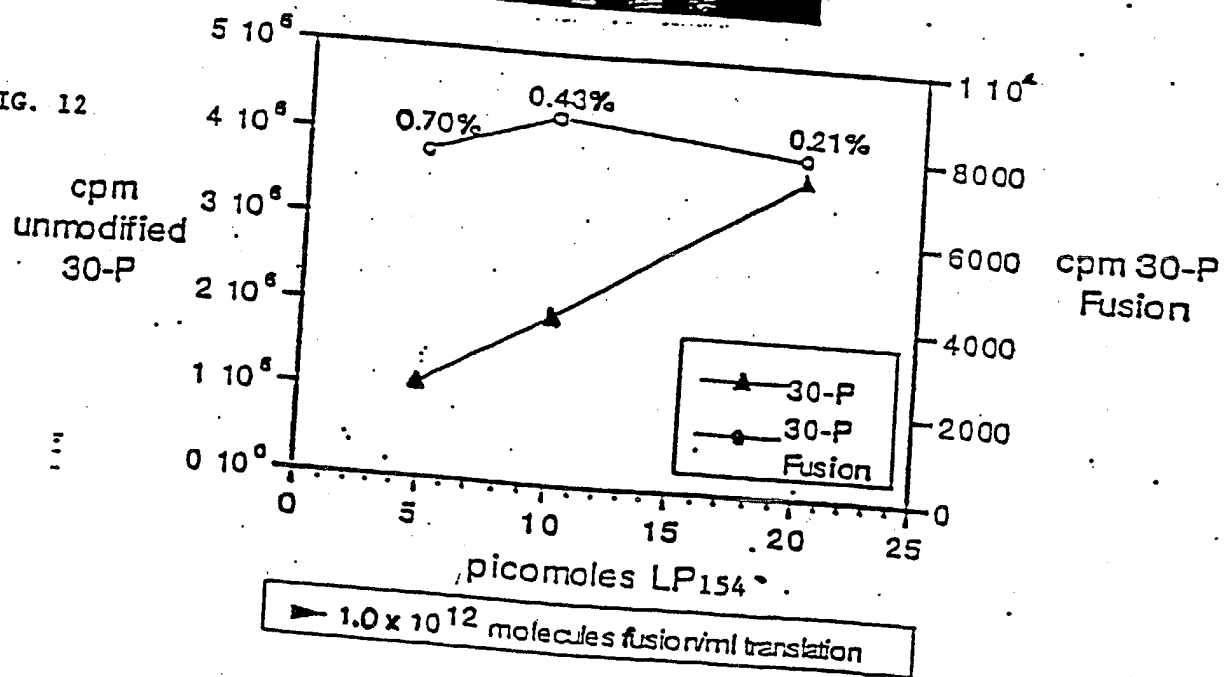


FIG. 13

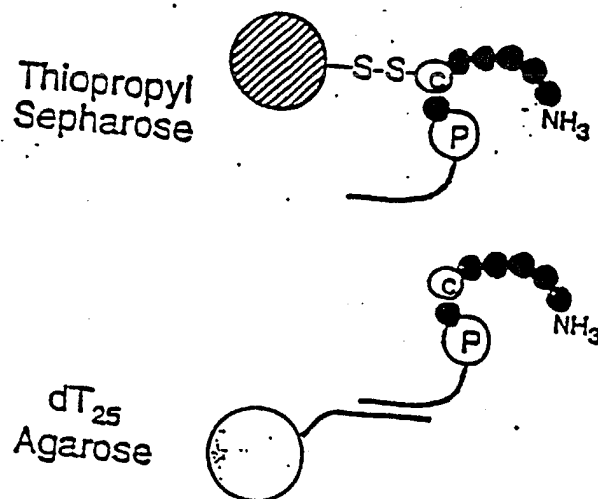
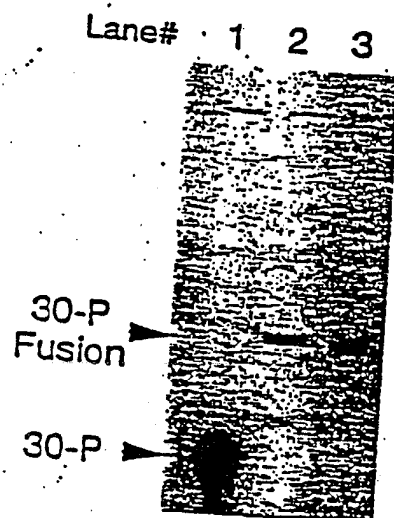


FIG. 14



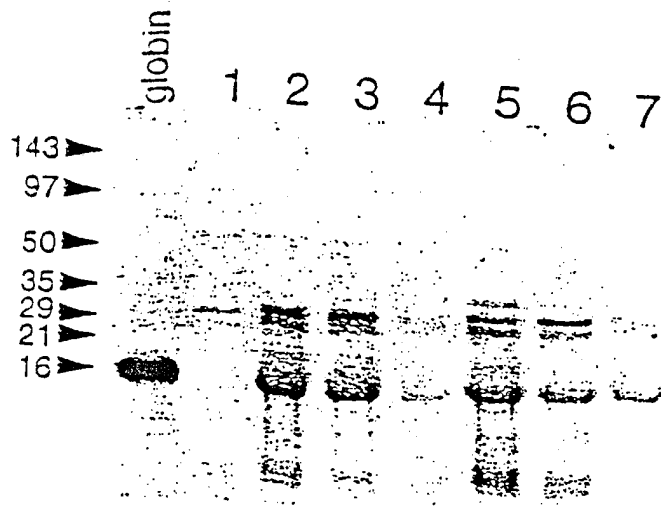


FIG. 15A

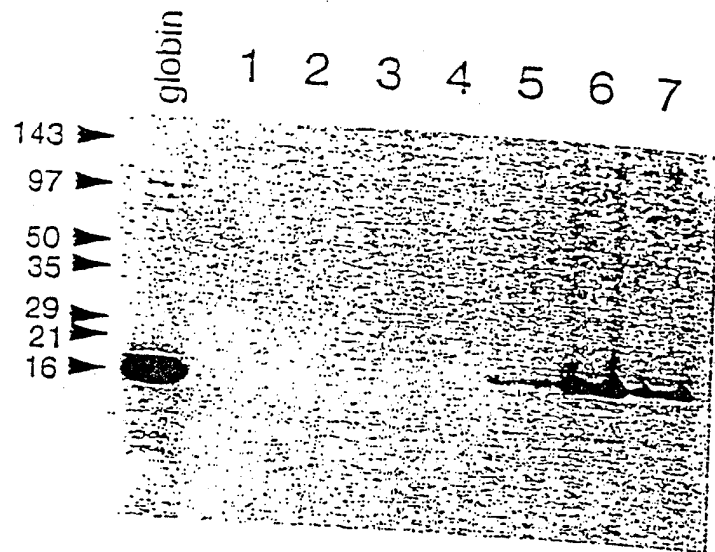


FIG. 15B

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FIG. 16 A

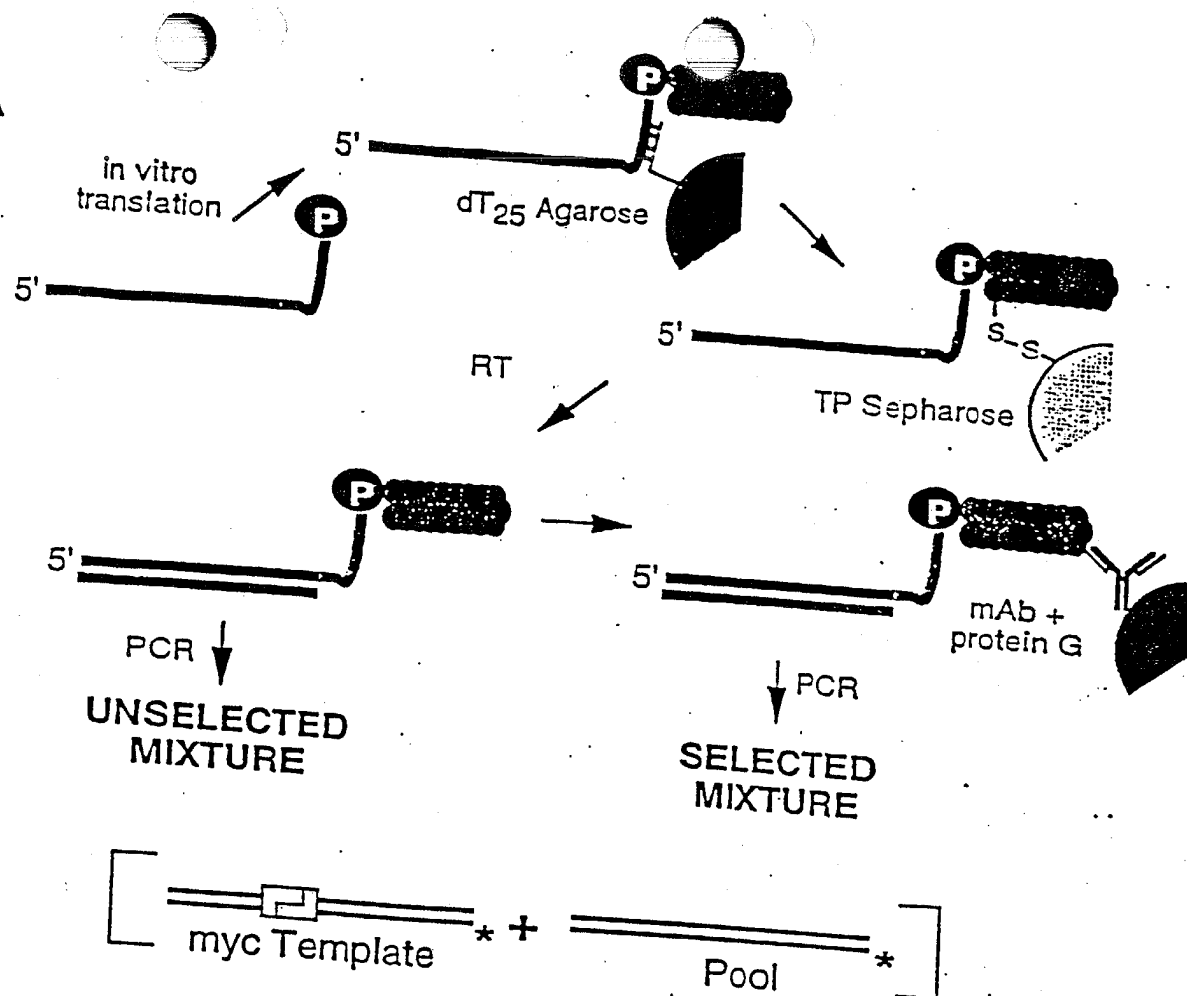


FIG. 16 B

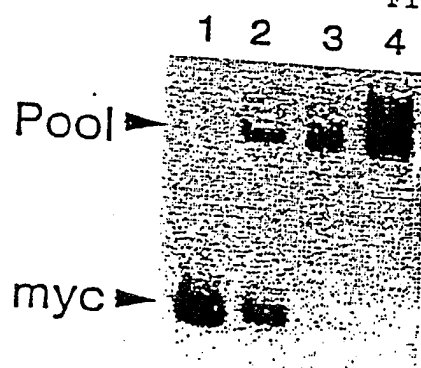
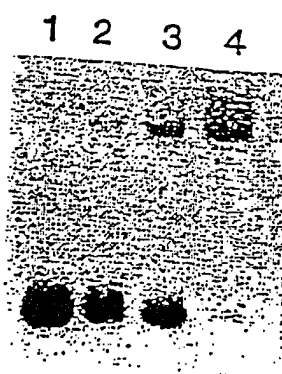


FIG. 16 C



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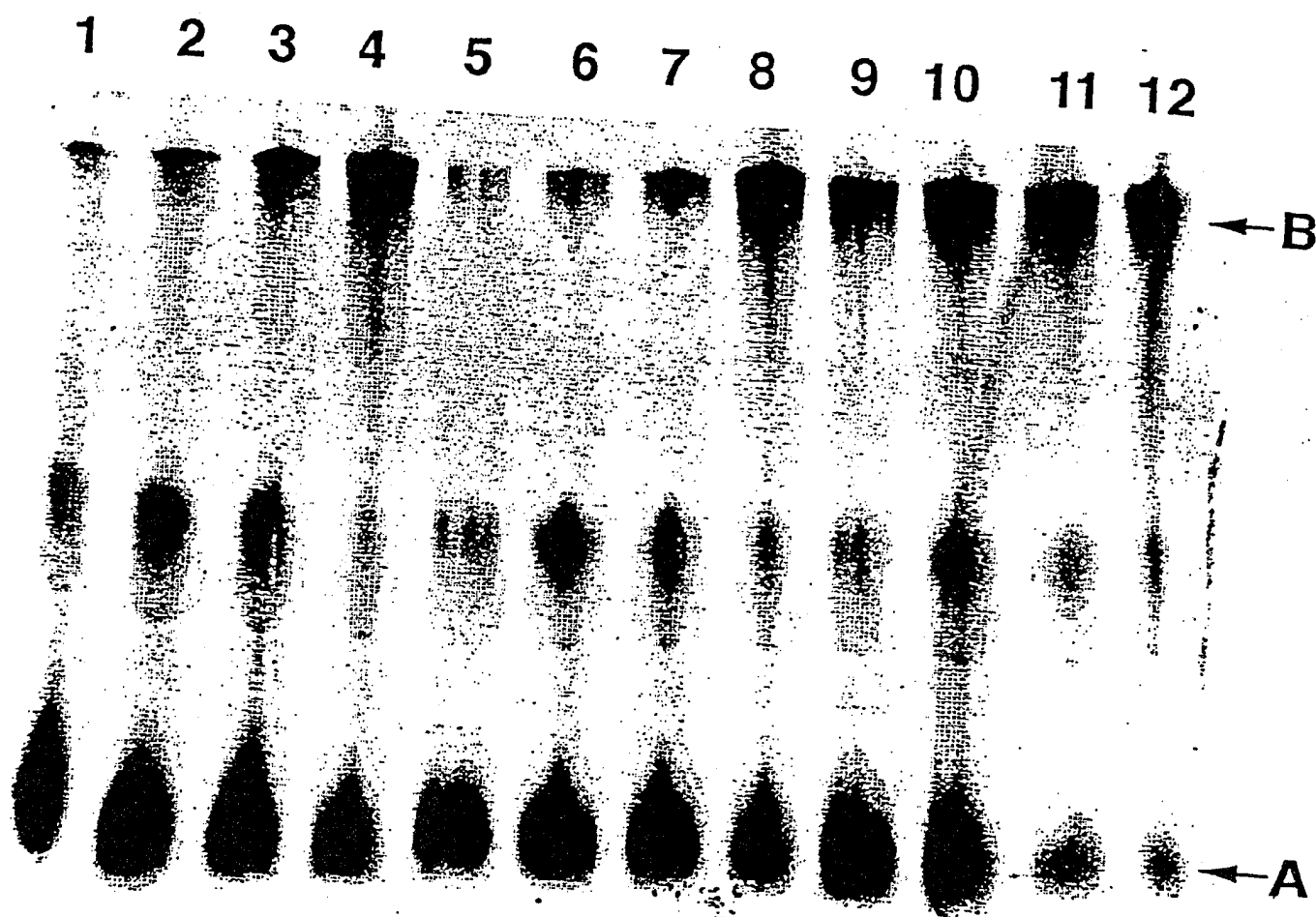


FIG. 17

1 2 3 4 5 6

mRNA-Peptide →

mRNA →

4.

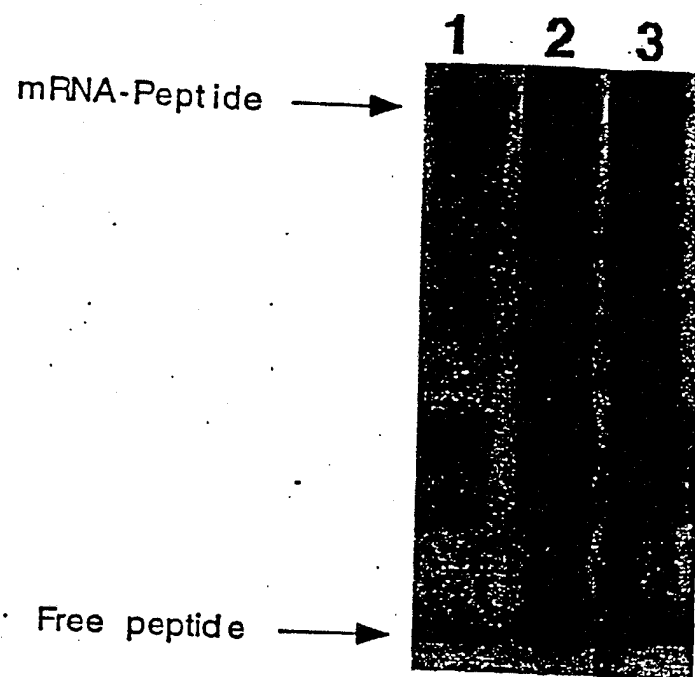


FIG. 19

FIG. 20

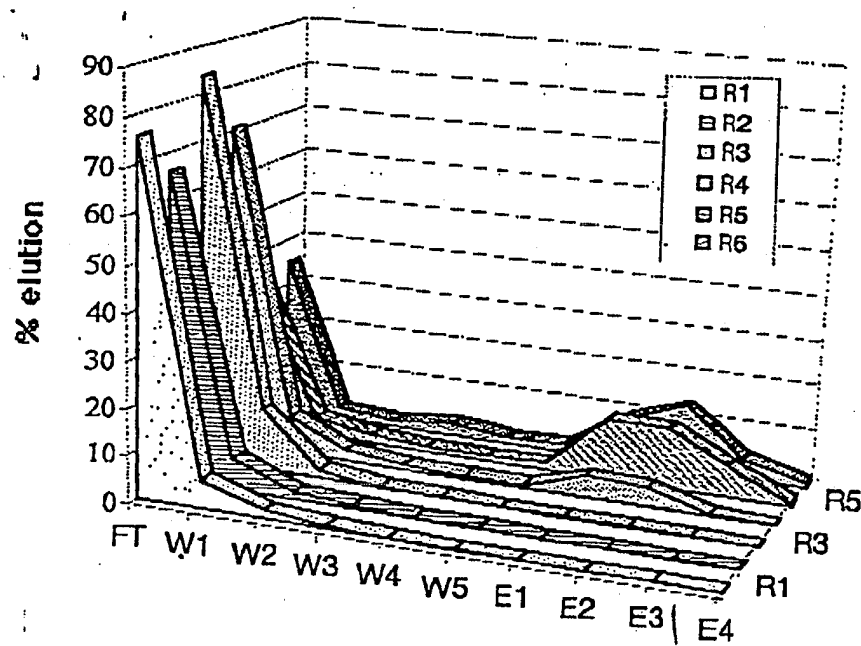


FIG. 21

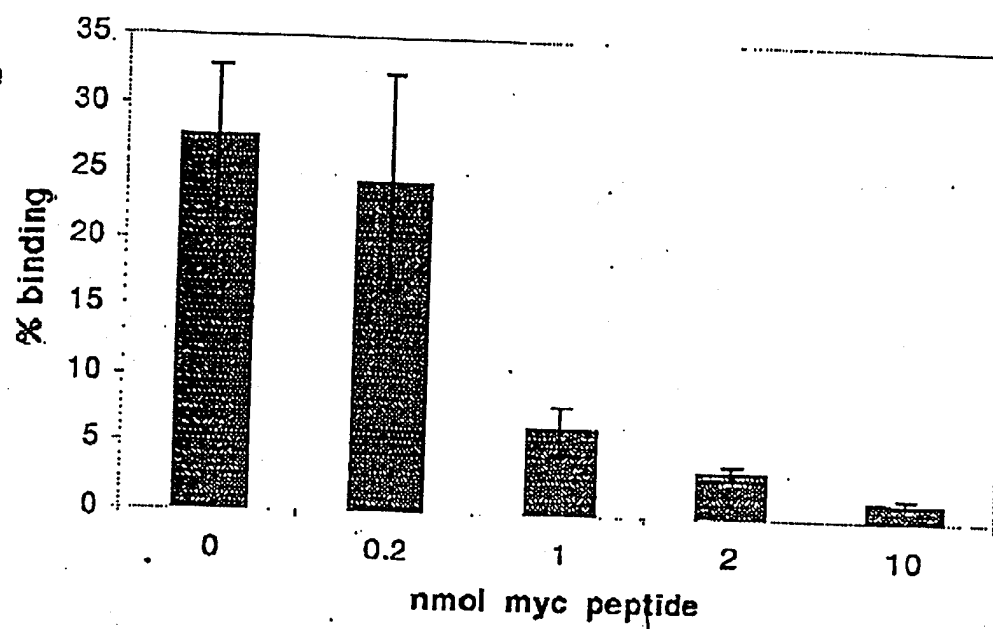


FIG. 22

c-myc epitope	E	Q	K	L	I	S	E	E	D	L
R6-51	C	A	S	V	L	S	E	R	E	C
R6-52	E	E	Y	L	V	S	E	Y	V	M
R6-53	R	Q	Y	L	I	S	E	Y	E	H
R6-55	L	Q	R	L	L	S	E	Q	M	F
R6-56	I	V	R	L	L	S	E	Y	H	M
R6-58	E	E	Y	L	L	S	E	Y	V	M
R6-60	M	Q	N	L	L	S	E	H	E	L
R6-61	T	M	D	L	L	P	E	H	Y	M
R6-63	E	Q	K	L	L	S	E	E	D	L
R6-66	D	M	M	L	L	S	E	K	E	I
R6-67	F	Q	A	L	L	A	E	E	E	I
R6-68	Q	R	V	L	L	S	E	F	W	L
consensus	X	Q/E	X	L	I	S	E	X	X	L/M

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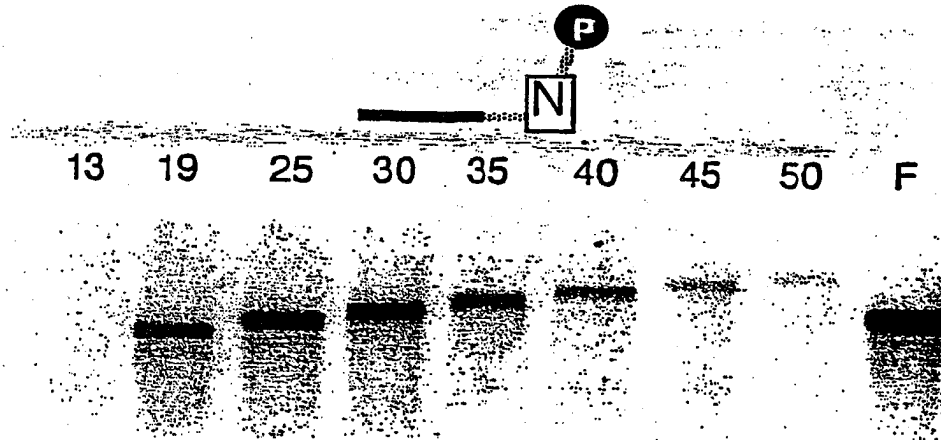


FIG. 23

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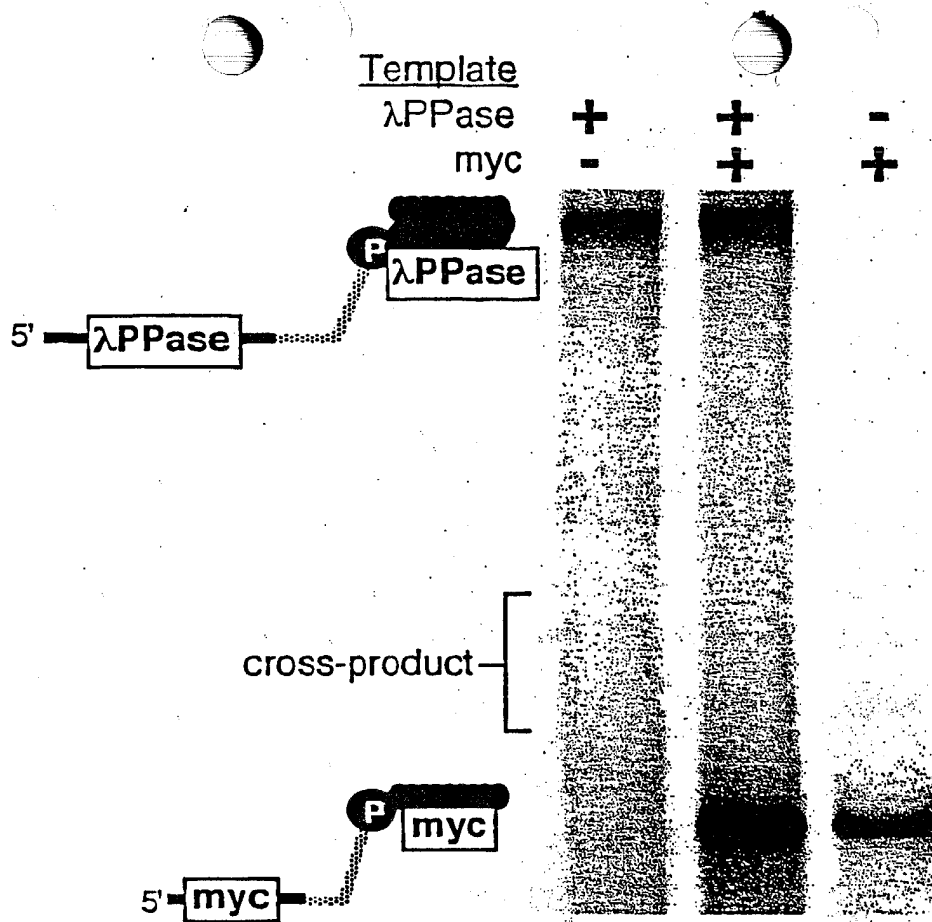


FIG. 24